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Sequence, tissue distribution, and chromosomal localization of mRNA encoding a human glucose transporter-like protein

(membrane protein/gene family/human chromosome 3)

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ABSTRACT **cDNA clones encoding a glucose transporter-like protein have been isolated from adult human liver and kidney cDNA libraries by cross-hybridization with the human HepG2/erythrocyte glucose transporter cDNA. Analysis of the sequence of this 524-amino acid glucose transporter-like protein indicates that it has 55.5% identity with the HepG2/erythrocyte glucose transporter as well as a similar structural organization. Studies of the tissue distribution of the mRNA coding for this glucose transporter-like protein in adult human tissues indicate that the highest amounts are present in liver with lower amounts in kidney and small intestine. The amounts of glucose transporter-like mRNA in other tissues, including colon, stomach, cerebrum, skeletal muscle, and adipose tissue, were below the level of sensitivity of our assay. The single-copy gene encoding this glucose transporter-like protein has been localized to the q26.1→q26.3 region of chromosome 3.**

The liver is an important site of glucose uptake and storage, and mobilization of glucose from this tissue helps to maintain glucose homeostasis (1). Studies using isolated hepatocytes indicate that glucose uptake by these cells occurs by a low-affinity, high-capacity facilitative glucose transport system that is not acutely modulated by insulin (2). The liver protein responsible for this activity has not been purified and little is known of its structure or the factors that regulate its biosynthesis. cDNA clones encoding the glucose transporter expressed in a human hepatoma cell line (HepG2) and the cognate protein from rat brain have been isolated and characterized (3, 4). mRNA encoding the HepG2 glucose transporter and corresponding rat protein has been identified in a number of tissues, suggesting that this protein is widely distributed and likely involved in glucose uptake by many different adult and fetal tissues, including fetal and neonatal liver (3–6). However, the levels of the mRNA encoding this transporter in adult liver are very low, suggesting that the HepG2 glucose transporter is not the main transport protein involved in glucose uptake by this tissue. Reasoning that there might be a family of related glucose transport proteins, we screened an adult human liver cDNA library using low-stringency hybridization conditions to identify clones that might cross-hybridize with the HepG2 glucose transporter cDNA.§ This strategy revealed cDNA clones encoding a 524-amino acid protein having a similar structural organization and 55.5% amino acid identity with the 492-amino acid HepG2/erythrocyte glucose transporter. mRNA encoding this protein, which we believe may be the liver glucose transporter, was also found in adult kidney and small intestine, although at lower levels than in adult liver; it was not detectable at the levels of sensitivity of our assay in colon, stomach, cerebrum, skeletal muscle, or adipose tissue. This putative liver-type glucose transporter is encoded by a gene that has been localized to the long arm of human chromosome 3.

MATERIALS AND METHODS

General Methods. Standard procedures were carried out as described in Maniatis *et al.* (7). Human tissues were obtained with institutional approval. Probes were labeled by nick-translation. For RNA transfer blots, 20 μg of total RNA was denatured with glyoxal and, after electrophoresis through a 1% agarose gel, transferred to a nitrocellulose filter. Standard hybridization conditions have been described (8). DNA sequencing was done by the dideoxynucleotide chain-termination procedure (9) after subcloning appropriate DNA fragments into M13mp18 or M13mp19. Both strands were sequenced.

cDNA Cloning. One million phage from a human liver cDNA library in λgt11 (Clontech, Palo Alto, CA, no. HL1001b) were screened with a ^{32}P-labeled 880-base-pair (bp) *Eco*RI–*Nco* I fragment (encoding amino acids 95–387) from the human HepG2 glucose transporter cDNA clone λhGT2 (10) using low-stringency conditions [37°C; 25% formamide, 0.75 M NaCl/75 mM sodium citrate, 2× Denhardt's solution (Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 20 mM sodium phosphate buffer (pH 6.5), 0.1% $NaDodSO_4$, 100 μg of sonicated, denatured salmon testes DNA per ml, 10% dextran sulfate, and 1×10^6 cpm of probe per ml; the filters were washed in 0.3 M NaCl/30 mM sodium citrate/0.1% $NaDodSO_4$ at room temperature and for 1 hr at 40°C before autoradiography]. Additional clones were identified in this liver cDNA as well as an adult human kidney cDNA library having inserts >2 kilobase pairs (kbp) (vector, λgt10; complexity, ≈250,000) (11) by hybridization under standard stringent hybridization conditions using *Eco*RI inserts from λhHTL-1 or -14 as probes.

Gene Mapping. The chromosomal localization of the liver-type glucose transporter gene was determined by hybridization of the ^{32}P-labeled insert from λhHTL-1 to Southern blots of *Bam*HI-digested DNA of each of 37 different human–mouse somatic cell hybrid cell lines. The regional localization was determined by hybridization of λhHTL-1 to metaphase chromosomes (10).

RESULTS

Isolation and Sequence of Glucose Transporter-Like cDNA Clones. Using low-stringency hybridization conditions, a

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§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03810).

fragment of the HepG2 glucose transporter cDNA hybridized to a large number of clones in an adult human liver cDNA library. Twenty clones were selected and of these 17 continued to hybridize with the probe during subsequent plaque purification. Digestion of purified DNA from these 17 recombinants with *Eco*RI revealed four λhHTL-1, -3, -13, and -14, having inserts of different sizes. The sequences of these clones indicated that they represented different portions of a mRNA encoding a protein related to the HepG2/erythrocyte glucose transporter (none coded for the HepG2 transporter itself); however, it was unlikely that any of these clones encoded the N terminus of the protein. Rescreening the adult human liver cDNA library revealed a single clone that provided an additional 10 bp at the 5′ end of the cDNA sequence. As RNA transfer blotting indicated the presence of this mRNA in adult kidney, a human kidney cDNA library was screened with the 5′ *Eco*RI insert from λhHTL-14. Seventeen clones hybridized with this probe and 6 of these also hybridized with a synthetic oligonucleotide based upon the sequence of 5′ end of λhHTL-14. Three of the 6 clones, λhHTL-210, -211, and -217, were different and their sequences included the liver cDNA sequence (Fig. 1).

The composite cDNA sequence of 3168 bp contains a single long open reading frame encoding a protein of 524 amino acids and molecular weight of 57,499 (Fig. 2) that has 55.5% identity with the HepG2/erythrocyte glucose transporter (Fig. 3). When conservative substitutions are considered, there is 67.9% amino acid similarity between these two proteins. There is 58.4% nucleotide identity between the coding regions of this cDNA and the HepG2/erythrocyte glucose transporter cDNA.

As noted in Figs. 1 and 2, cDNAs were isolated having poly(A) tracts at two different sites, following nucleotides 2563 (noted by the asterisk in Fig. 2) and 3168. Neither of these poly(A) tracts is preceded by the typical poly(A) signal, AATAAA (13). Thus, the 3′ untranslated region of human glucose transporter-like mRNA is 953 or 1558 bases; moreover, as indicated below, it is likely that some transcripts may have a 3′ untranslated region of about 3.7 kb. Three nucleotide differences were also observed between the various cDNA sequences (Fig. 2), of which one results in a proline → leucine replacement at amino acid 68.

Tissue Distribution of Glucose Transporter-Like mRNA. Transfer blotting of RNA prepared from various adult human tissues revealed hybridization to transcripts of 2.8, 3.4, and 5.4 kb (Fig. 4). The 2.8- and 3.4-kb transcripts likely correspond to the two types of cDNAs, differing in the length of the 3′ untranslated region that were isolated and characterized above. Although undetermined, we suspect that the 5.4-kb transcript differs from the others in having a much longer 3′ translated region. These transcripts were most abundant in liver and present in lower amounts (by a factor of ≈2–3) in kidney and small intestine (upper jejunum) (Fig. 4). The amounts of mRNA present in term placenta, colon, stomach, skeletal muscle (semitendinous), cerebrum, gall bladder, and subcutaneous fat were below the levels of sensitivity of our assay. Glucose transporter-like mRNA was also present in three different hepatomas but was undetectable in a colon carcinoma or leiomyosarcoma. We have also detected this mRNA in HepG2 cells (data not shown), although its relative abundance in these cells is lower by a factor of ≈10 than that encoding the HepG2/erythrocyte glucose transporter.

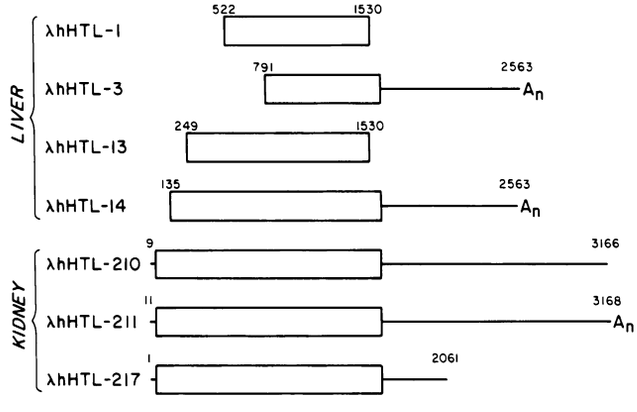

FIG. 1. Schematic representation of human glucose transporter-like cDNA clones studied. The protein coding portion of each clone is indicated as a box. The clones isolated from liver or kidney cDNA libraries are noted. The numbers correspond to the 5′ and 3′ ends of the cDNA inserts relative to the composite cDNA sequence. Clones having poly(A) tracts are indicated.

Chromosomal Localization of the Human Glucose Transporter-Like Gene. The chromosomal assignment of the human glucose transporter-like gene, designated *GLUT2* [the HepG2 glucose transporter gene has been designated as *GLUT* (10)], was determined from analysis of its segregation in a panel of mouse–human somatic cell hybrids as well as by *in situ* hybridization to metaphase chromosomes. The *Eco*RI insert from λhHTL-1 hybridized to two human *Bam*HI fragments of 12 and 6.7 kbp, which could be readily distinguished from the single mouse DNA fragment of 15 kbp (data not shown). In addition, this probe hybridized to a single human *Eco*RI fragment of 8.3 kbp (K. Xiang and H.F., unpublished), indicating that there is only a single copy of the *GLUT2* gene in the human. Hybridization to DNA prepared from a panel of 37 cell hybrids demonstrated that the two human DNA fragments were only present in those hybrids retaining the long arm of human chromosome 3 (data not shown but available upon request). Moreover, the absence of hybridization to human DNA fragments in two hybrids having a 3/X translocation suggested that the human glucose transporter gene was in the region of 3q21→3qter. *In situ* hybridization to metaphase chromosomes confirmed the localization to the long arm of human chromosome 3 and indicated that the gene is in the q26.1→q26.3 region of this chromosome (Fig. 5).

DISCUSSION

Although the liver has a major role in the regulation of glucose homeostasis, the proteins involved in the transport of glucose by this tissue are poorly characterized. We have isolated and sequenced adult human liver (and kidney) cDNAs that encode a 524-amino acid protein that has 67.9% similarity (55.5% identity) with the HepG2/erythrocyte glucose transporter. This value is much greater than the 30% identity observed between the sequence of the HepG2 glucose transporter and those of the arabinose-H^+ and xylose-H^+ symporters, membrane transport proteins of *Escherichia coli* that share sequence and structural similarity with the mammalian glucose transporters. Because of its homology with the HepG2/erythrocyte glucose transporter as well as the relatively high levels of its mRNA in liver, we believe that the glucose transporter protein described here may be the liver glucose transporter, although we cannot exclude a role for this protein in the uptake of another sugar. It appears likely though that there is a family of proteins involved in sugar uptake in mammals, just as in *E. coli* (14).

Mueckler *et al.* (3) have presented a model for the topology of the membrane-bound HepG2 glucose transporter and propose that the protein spans the plasma membrane 12 times and has an extracellular loop between the first and second transmembrane segments as well as a large hydrophilic intracellular loop between transmembrane segments six and seven. Our analysis of the primary structure of the glucose

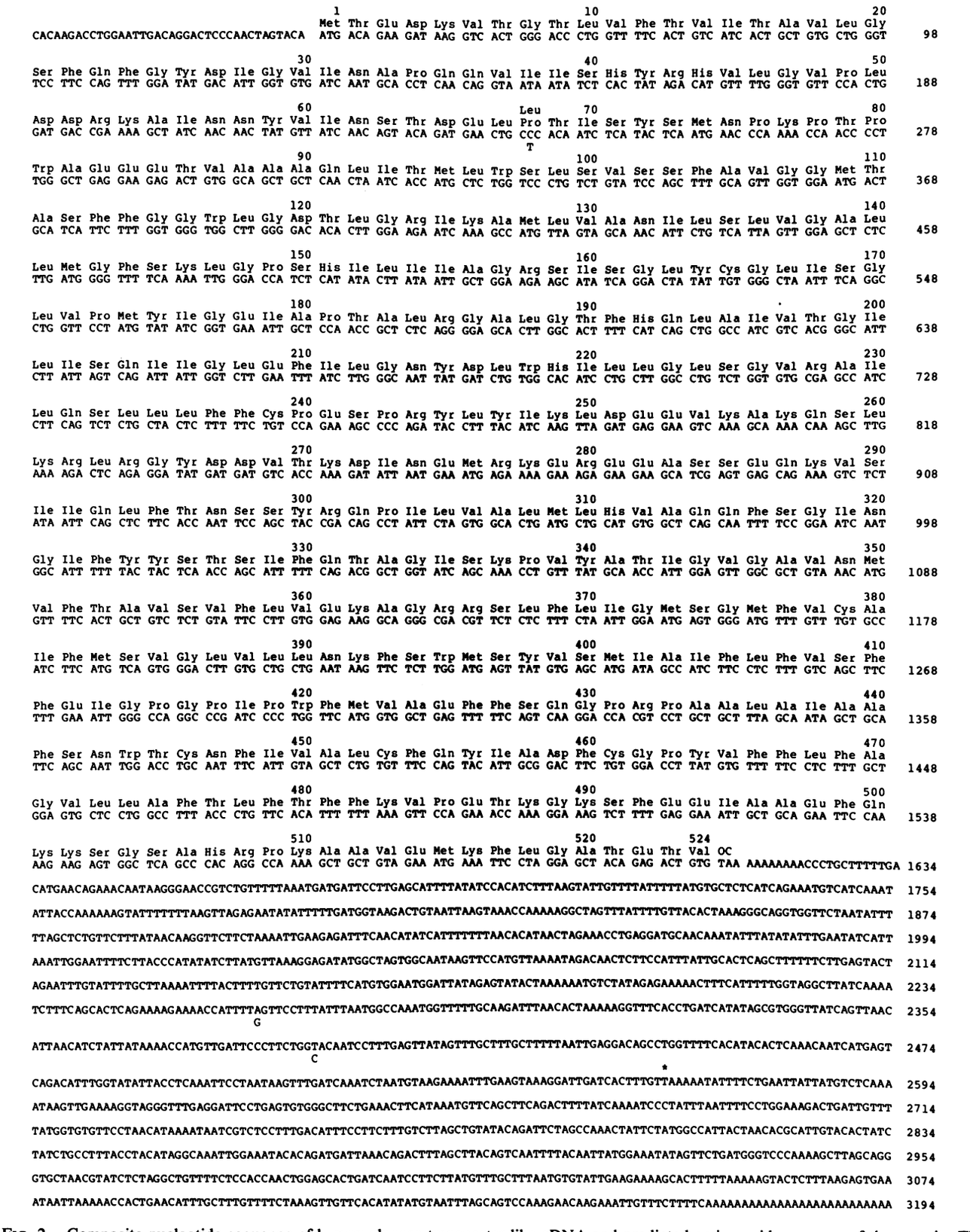

FIG. 2. Composite nucleotide sequence of human glucose transporter-like cDNA and predicted amino acid sequence of the protein. The number of the nucleotide at the end of each line is indicated. Sequence differences between the various clones examined are noted. The asterisk in the 3′ untranslated region indicates the position of the poly(A) tract in the liver cDNA clones λhHTL-3 and -14.

transporter-like protein suggests that it could be organized in a similar fashion. Although the sequences and possible topology of these two proteins are similar, there are also several noteworthy differences. Possibly the most significant difference is in the size and sequence of the extracellular loop; the glucose transporter-like protein has an additional 34 amino acids in this segment. In addition, the sequences of the C-terminal 28 residues of these two proteins are not well conserved.

The putative liver-type glucose transporter sequence has three possible sites for asparagine-linked glycosylation (15) at Asn-62, -297, and -443. The first site is predicted to be in an

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                                                    TM 1
Liver     1 M::TEDKVTGTLVFTVITAVLGSFQFGYDIGVINAPQQVIISHYRHVLGVPLDDRKAINNYVINSTDEPLTI
            *  |  *|** *|  *  ***** ****  ******* **   *        |
HepG2     1 MEPSSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQTWVHRYGESILPTT::::::::::::
                                                                 TM 2                TM 3
Liver    71 SYSMNPKPTPWAEEETVAAAQLITMLWSLSVSSFAVGGMTASFFGGWLGDTLGRIKAMLVANILSLVGALLM
                                 |* ******  * ****  **  *        **   **| *|*  * *|**
HepG2    61 ::::::::::::::::::::::LTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLLAFVSAVLM
                                                TM 4                TM 5
Liver   143 GFSKLGPSHILIIAGRSISGLYCGLISGLVPMYIGEIAPTALRGALGTFHQLAIVTGILISQIIGLEFILGN
            ****** *  ||* ** * *|**** |* ****|**| ********** *** ** **** *| **| *|**
HepG2   111 GFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQLGIVVGILIAQVFGLDSIMGN
                                    TM 6
Liver   215 YDLWHILLGLSGVRAILQSLLLFFCPESPRYLYIKLDEEVKAKQSLKRLRGYDDVTKDINEMRKEREEASSE
             *** |** |   | *|** ||* *******|* *   ** |**  **|***  *** *||**| *      *
HepG2   183 KDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEENRAKSVLKKLRGTADVTHDLQEMKEESRQMMRE
                                                TM 7                TM 8
Liver   287 QKVSIIQLFTNSSYRQPILVALMLHVAQQFSGINGIFYYSTSIFQTAGISKPVYATIGVGAVNMVFTAVSVF
             **|*| **    ******|*||* | ** **** |******** **|  ******* * **  ** **|*
HepG2   255 KKVTILELFRSPAYRQPILIAVVLQLSQQLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLF
                                    TM 9                TM 10
Liver   359 LVEKAGRRSLFLIGMSGMFVCAIFMSVGLVLLNKFSWMSYVSMIAIFLFVSFFEIGPGPIPWFMVAEFFSQG
            |**|****|* ***| **  *** *|| * **    ****|*||*** ** ***|********|*** ****
HepG2   327 VVERAGRRTLHLIGLAGMAGCAILMTIALALLEQLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQG
                                        TM 11               TM 12
Liver   431 PRPAALAIAAFSNWTCNFIVALCFQYIADFCGPYVFFLFAGVLLAFTLFTFFKVPETKGKSFEEIAAEFQKK
            *****|*|* ***** **** |****|   ****** |*  |*| * |**|********||*|***  *
HepG2   399 PRPAAIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDEIASGFRQG

Liver   503 SGSAHRPKAAVEMKFLGATETV 524
              *          ***  *
HepG2   471 GASQSDKTPEELFHPLGADSQV 492
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FIG. 3. Comparison of the sequences of human glucose transporter-like and HepG2 glucose transporter proteins. Amino acids are indicated by their single-letter abbreviations. Asterisks denote identical residues and lines indicate chemically similar amino acids. Gaps introduced to generate this alignment are represented by colons. The 12 predicted membrane-spanning regions (TM 1–12) (3) are underlined. Residue 152 in the HepG2 sequence is phenylalanine in Mueckler *et al.* (3) and leucine in the gene and an independently isolated cDNA clone λhGT2 (12).

extracellular loop and thus might be glycosylated. The HepG2 glucose transporter also has a site in this loop that is likely glycosylated. The other two sites are predicted to be in an intracellular loop and membrane-spanning segment, respectively. There is also a potential cAMP-dependent protein kinase phosphorylation site (16), Lys-Lys-Ser-Gly-Ser (residues 501–505, Fig. 2) in the C-terminal portion of the protein. This site is predicted to be located on the cytoplasmic side of the plasma membrane in a region whose sequence is poorly conserved between the glucose transporter-like protein described here and the HepG2 glucose transporter (Fig. 3) (the HepG2 glucose transporter sequence does not have a similar phosphorylation site in the corresponding region or elsewhere in the protein).

The tissue distribution of glucose transporter-like mRNA is quite different from that of HepG2 glucose transporter mRNA. We have only observed significant expression of glucose transporter-like mRNA in adult liver, kidney, and small intestine (jejunum). By contrast, the highest levels of mRNA encoding the HepG2-type protein (3–6) are present in term placenta and brain with slightly lower levels in kidney and colon and very low levels in a number of other tissues, including stomach, small intestine, gall bladder, subcutaneous fat, and skeletal muscle. We have been unable to detect HepG2 glucose transporter mRNA in our adult liver RNA preparations. The low levels of transcripts for both trans-

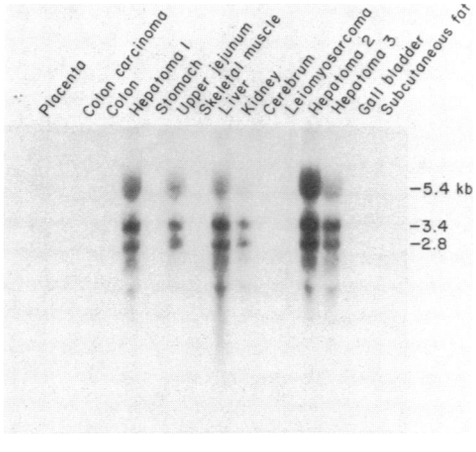

FIG. 4. Expression of glucose transporter-like mRNA in various adult human tissues and tumors. Twenty micrograms of total RNA was denatured with glyoxal, separated by agarose gel electrophoresis, and blotted onto a nitrocellulose filter. The filter was hybridized with the nick-translated insert from λhHTL-14 spanning nucleotides 135–1530. The sizes of the hybridizing transcripts were determined by comparison with the mobility of the DNA fragments in a *Hin*dIII digest of λ DNA.

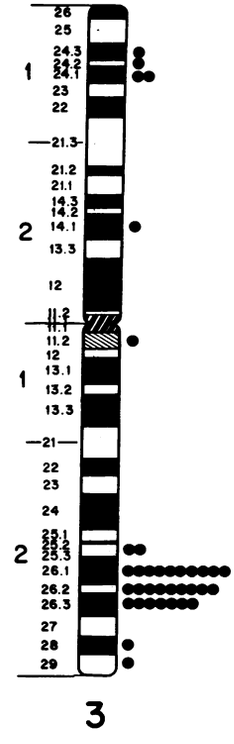

FIG. 5. Ideogram of human chromosome 3 showing silver grain distribution after hybridization with λhHTL-1 probe. Seventy-two percent of the grains on chromosome 3 are localized in the q26.1→q26.3 region. No other human chromosome demonstrated a grain distribution above background.

porters in skeletal muscle and adipose tissue are rather surprising and suggest that there may be other proteins that are responsible for glucose uptake by these tissues. It is also interesting that some tissues express several different glucose transporter/glucose transporter-like proteins—e.g., small intestine and kidney [these tissues also express the structurally unrelated Na^+/glucose cotransporter (17)]—but it is unknown if these transporters are coexpressed in the same cell in these tissues. However, as HepG2 cells express glucose transporter and glucose transporter-like mRNA, both proteins might be present in the same cell in some instances.

Glucose uptake by mammalian tissues may be mediated by a family of related proteins. The genes encoding two such proteins are located on different chromosomes. We have previously mapped the HepG2 glucose transporter gene to human chromosome 1p35→p31.3 (10), and the results presented here localize the gene encoding a related protein to human chromosome 3q26.1→q26.3. The strategy that we have used to isolate cDNA clones encoding the putative adult liver glucose transporter should also be applicable for screening other tissues for sequences homologous to these two transporters. We expect that such studies will reveal other related proteins. In addition, the expression in a well-defined heterologous system of cDNAs encoding each of these proteins will facilitate a critical analysis of their properties.

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